Impact Of Viral Infection on Absorption And Scattering Properties of Marine Bacteria And Phytoplankton

James Vaughn
Dept. of Microbiology
11 Hills Beach Rd.
Biddeford, ME 04005
Phone: (207)283-0171 Fax: (207)283-3249 email: jvaughn@mailbox.une.edu

William M. Balch
Bigelow Laboratory for Ocean Sciences
P.O. Box 475, McKown Point
W. Boothbay Harbor, ME 04575
Phone: (207) 633-9600 Fax: (207) 633-9641 e-mail: bbalch@bigelow.org

James Novotny
Dept. of Microbiology
11 Hills Beach Rd.
Biddeford, ME 04005
Phone (207)283-0171 Fax: (207)283-3249 e-mail: jnovotny@mailbox.une.edu

AWARD #: N00014-99-10645

LONG-TERM GOALS

The long range goal of our research is to document the role of viruses in light scattering in the sea. The current DEPSCoR project is a joint venture between the University of New England and its affiliate, Bigelow Laboratory.

OBJECTIVES

The objectives of this work are to: 1) define the typical time scale for the shift in inherent optical properties (a, b, c, and bb) associated with viral infection of marine bacteria and phytoplankton (as compared to non infected control populations), 2) define the maximum possible rates of shift in inherent optical properties (a, b, c, and bb) under conditions of high virus multiplicity of infection, and 3) define the concurrent changes in 0.03- 100 μm size spectra associated with viral infection of marine bacteria and phytoplankton.

APPROACH

This work involves a significant number of preparatory laboratory experiments leading to several large-scale mesocosm experiments to be conducted off of the Bigelow Dock (West Boothbay Harbor, ME). These will provide adequate sample volume to make all size spectrum, bb, and cell count measurements, plus the AC-9 measurements of a and c (which require larger volumes). Scattering will be calculated as the difference between c and a. By using large volumes with known host and controlled virus addition, we can be assured that 1) inoculated viruses are host specific, 2) host culturing conditions are optimal for virus assays, 3) host growth conditions are sufficiently understood

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 1999		**00-00-1999 to 00-00-1999**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Impact Of Viral Infection on Absorption And Scattering Properties of Marine Bacteria And Phytoplankton	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **University of New England,Department of Microbiology,11 Hills Beach Rd,Biddeford,ME,04005**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**4**	

from preliminary experiments in aqueous media, 4) initial viral concentrations are easily set, 5) bottle effects are minimized because of the large volumes, and 6) mesocosms will provide a realistic scale for observing viral infection dynamics in blooms of marine organisms. In our review of the literature, we have observed many cases where strict controls were not run such that results were too ambiguous to interpret .

WORK COMPLETED/RESULTS

The first three months of the project have been devoted to the acquisition of culturable marine virus/host systems which will be used in laboratory scale and mesocosm experiments. Work to date has focused on the isolation of viruses of non-photosynthetic marine bacteria using the methods described below. Isolate source waters have included Saco Bay, West Boothbay Harbor, Portland Harbor, and the Gulf of Maine (samples collected from Maine and Nova Scotia coastal areas). As of this writing, ten new phage/host systems have been isolated. Appropriate descriptive studies (eg. Phage growth curves, host identification, electron microscopy studies, etc.) are under way.

Isolation and Enumeration of Marine Bacteriophage and Host Cells
Briefly, candidate host bacteria were isolated using a membrane filtration method. Membranes were placed on pads containing 2216 Marine Broth and incubated overnight at 25C. Resulting colonies were "picked" and grown overnight as pure cultures in 2216 broth. Phage isolation involved an enrichment technique. Overnight cultures (100 ml) of candidate host cultures were concentrated by centrifugation (5000 x g/15 min), resuspended in 5 ml of 2216 broth and inoculated into 500 ml flasks containing triple strength 2216 broth (100 ml) and 300 ml of the original water sample from which the candidate host had been isolated. Enrichment flasks were incubated (25^{o}C) with shaking for 1-3 days. Following incubation, 5 ml aliquots from each enrichment were filtered (0.22 μm), "spot tested" on lawns of original host cell and observed for the development of typical virus plaques over a 24-48h period. New phage and host cell stocks were then stored in 1ml aliquots at –74^{o}C to await further testing. At present, new phage isolates and their host cells are designated based upon their isolation site: Saco River (high tide), K-04 & 05; Saco Bay (high tide), LS-02, 04 & 05; West Boothbay Harbor, WBH-02->05.

Bacteriophage Growth Characteristics
To date, all phage isolates have undergone at least one growth experiment. Briefly, 3h cultures of host bacterium (~ 10^8 colony forming units [cfu]/ml) were inoculated with appropriate phage (~10^7 pfu/ml). One milliliter sample aliquots, collected at ten minute intervals over a 2-3 hr growth period, were diluted and assayed for viable phage numbers using a plaque assay technique (Adams, 1959). To date, six of the nine new phage isolates have demonstrated very strong growth characteristics, with 2 to 3 log increases in titer over the course of each growth experiment. The average burst time (ie. time for a single virus replication cycle) has been approximately 60 min.

IMPACT/APPLICATIONS

These results represent important preliminary steps in furthering our understanding of the role of viruses in light scattering in the sea. Experiments outlined below will assess the impact of replication of the new phage isolates on the optical properties of their specific host cells.

TRANSITIONS

We are preparing to examine the impact of newly isolated viruses of marine non-photosynthetic bacteria on bacterial volume scattering.

Optical Measurements
A Dawn Laser Light Scattering Photometer will be used for measurement of the volume scattering function, and calculation of the backscattering coefficient (b_b). This instrument makes 400 measurements of the phase function each second and averages the data over any pre-set time period. WMB currently owns a Wyatt Technologies Dawn Photometer equipped with argon ion laser light source (512 nm) with capability of discrete or continuous flow-through measurements. As part of a previous ONR-funded study, the instrument was upgraded from a helium neon laser. The upgrade provides light at a wavelength more relevant to the ocean. If required, light scattering measurements can be directly coupled to size measurements from a field-flow fractionator. Thus, for each size fraction separated by the field-flow fractionator, we produced an average volume scattering function and bb estimate.

Effects of Virus Growth on Optical Properties of Host Cell Suspensions
Three hour broth cultures of appropriate host cell propagated in 2216 Marine Broth will be inoculated with its specific phage. Following a 5 min. adsorption period, suspensions will be diluted (1:1000) into cuvettes and incubated (25^{o}C) for 3 to 4h. At intervals of 10 to 15 min, cuvettes will be placed in the Dawn for optical measurement. At the same time, 1 ml aliquots will be removed, diluted and assayed for viable virus and host concentrations. Separate aliquots will also be collected for direct microscopic assay using the SYBR Green method (Noble and Fuhrman, 1998).

OtherPlanned Studies
Upon completion of studies with non-photosynthetic marine bacteria and their viruses, our work will shift to the isolation and purification of viruses of photosynthetic marine bacteria. We are currently propagating several species of Synecococcus and other photosynthetic bacteria, obtained from the Bigelow Provasoli-Guillard Center for the Culture of Marine Phytoplankton. These will be eventually be used in attempts to recover species-specific virus strains from Maine coastal waters. Once isolated, virus and host cultures will be used in optical experiments similar to those described above. Also envisioned for Yr 1 will be a series of descriptive studies of new virus isolates including purification, concentration, Electron Microscopy, and nucleic acid typing. It is expected that purified, isolate concentrates will be subjected to the type of optical analyses described in our first ONR project (Optical Properties of Viruses). While not the principle focus of the current project, we endeavor to expand our virus optics database whenever possible.

RELATED PROJECTS

This DEPSCoR work is a joint venture between the University of New England and its affiliate Bigelow Laboratory. Collaborative relationships are maintained with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics. This work is the logical outgrowth of a recently completed DEPSCoR project (N000149801999).

REFERENCES

Adams, M. H. 1959. Bacteriophages. Interscience. New York, NY.

Bachrach, U. And A. Freidman. 1971. Practical procedures for the purification of bacterial viruses. Appl. Microbiol. 22: 706-715.

Noble, R. T. and J. A. Fuhrman. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquatic Microbial Ecology 14: 113-118.